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Pilot-scale ultrasound-assisted extraction of protein from soybean processing materials shows it is not recommended for industrial usage

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ABSTRACT

Unit operations to enhance protein extraction within the food industry are vital to improve current processes, especially for cost reductions and sustainability. Here a study of ultrasound-assisted extraction (UAE) from soy slurry and okara produced at pilot-scale and further processed using a lab or pilot-scale probe system is presented. Confocal imaging and particle size measurements were used to study the physical effects of UAE on these soy processing materials. Ultrasound at pilot-scale was infeasible for soy slurry, in contrast to lab-scale. UAE from okara solution significantly increased protein yield by 4.2% at pilot-scale ($p < 0.05$). Okara solution flow rate and okara concentration also significantly improved the protein extraction yield. During lab-scale sonication of okara solution, a greater energy intensity resulted in a higher yield of up to 40% after 15 min treatment. Considering total extraction yields at pilot-scale during soybase production, ultrasound is not considered viable for industrial processing.

1. Introduction

Soybeans are a source of 'complete' protein, providing the body with all of the essential amino acids that humans are unable to synthesise. Soybeans range in composition and their use is dependent on their desired function; for soymilk preparation, soybeans are chosen with a high protein content, compared to those utilised for oil extraction. In terms of resources: less energy, water and land is required to provide the world with sufficient protein from plant-based sources compared to animal-based (Aiking, 2011). For these benefits to be realised, the processing of raw materials to provide the final products needs to be efficient. During current soymilk processing plants, a significant portion of the available protein enters the waste stream currently utilised as animal feed (O'Toole, 1999). A more sustainable extraction of components is required using a green technology to make soymilk manufacture more profitable on social, economic and environmental levels.

The soybean microstructure is complex. Within the storage cells of the soybean, protein is organised in 5–20 μm protein bodies, surrounded by a cytoplasmic network containing oil bodies in the size range of 0.2–0.5 μm stabilised by proteinaceous oleosins (Rosenthal et al., 1998). In order to solubilise components inside the cells, the solvent needs to be in direct contact with those components, this is most easily facilitated by cell disruption. During soymilk production, soybeans are milled under hot (>80 °C), alkaline (pH 8+) conditions to solubilise protein, as well as inactivate the enzyme lipoxygenase and trypsin inhibitors (Vishwanathan et al., 2011). Insoluble materials are removed from the slurry using centrifugation; this gives two streams: soybase, the precursor for soymilk, and a waste stream, termed okara. The okara has been shown, using confocal laser scanning microscopy (CLSM) (Preece et al., 2015), to contain both intact cells and insoluble protein in the continuous phase. It would obviously be valuable to increase the process yield by breaking up a higher proportion of the cells.

Ultrasound has been widely studied in the food industry for aiding the extraction of components of interest from plant sources (Chandrapala et al., 2013; Chemat et al., 2011; Esclapez et al., 2011; Patist and Bates, 2008; Shirsath et al., 2012; Vilkhu et al., 2008). Ultrasound has been utilised and shown promise as a green technology within the field of extraction, reasons including reductions in extraction times, solvent use and more effective energy utilisation, as well as improving product quality (Chemat et al., 2017; Jacotet-Navarro et al., 2016; Li et al., 2013; Sicaire et al., 2016). The success of ultrasound is attributed to the cavitation phenomenon.

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Conditions attributed to cavitation assist the solubilisation of materials into the liquid medium, enhancing the extraction. Upon asymmetric bubble collapse, liquid jets are formed which can disrupt cells upon contact with cell walls (Li et al., 2004; Shirsath et al., 2012), causing the release of intracellular compounds. A detailed overview of the mechanisms responsible for the enhancement of extraction yield from plant materials associated with ultrasound is described by Chemat et al. (2017). For ultrasound to be considered as a green technology, the greenhouse gas emissions resulting from the treatment should be lower in comparison to the greenhouse gases which are saved by the reduction in soybeans required.

Soy-based studies are present examining the effects of ultrasound on the extraction of various compounds. For protein and sugar extraction, one such study by Karki et al. (2010) showed the application of ultrasound (20 kHz, ≤2 min treatment) improved the extraction yields from hexane-defatted soy flakes at lab-scale. Protein functionality improvement from soy protein isolate (SPI) and concentrates (SPC) has also been reported with positive results in protein solubility and particle size reduction (Lee et al., 2016). Some studies on ultrasound-assisted extraction (UAE) from soy-based systems as the starting material exist, yet direct extraction from the soybean has largely been neglected. Preece et al. (2017b) showed that ultrasound (20 kHz, ≤15 min) improved protein extraction yield by up to 21% & 25% for directly from soybeans in a lab-scale system, through slurry treatment and okara treatment on samples prepared at lab-scale, respectively (Preece et al., 2017b). This research (Preece et al., 2017b) partially supported the patent of Wijngaard and Zuidam (2014), where improvements in extraction yields were only observed during okara solution sonication. During the preparation of soy-based beverages, direct extraction of proteins, oils and other alkali-soluble components by wet milling from soybeans is commonly used during factory-scale manufacture (Vishwanathan et al., 2011). UAE of soy protein has been studied previously (Moulton and Wang, 1982) under continuous conditions on pilot-scale, using defatted soy flakes as the starting material. However, this study was carried out more than 30 years ago, and key experimental data, such as particle size measurement and visualisation of the microstructure, were neglected. Direct extraction from soybeans are rarely studied at a lab-scale, and no pilot-scale studies were found in the literature.

Pilot-scale use of ultrasound has been documented for a limited number of food systems, other than soy protein extraction. Pingret et al. (2012) showed an improvement of 30% extraction of polyphenols was achievable using ultrasound-assistance (20 kHz, 40 °C, 40 min) versus conventional extraction from apple pomace in a 30 L tank. Another study focused on waste stream valorisation; phenolic compounds were extracted from maritime sawdust waste using UAE (25 kHz, 40 min) with an increased phenolic yield of 30% compared to conventional maceration on a pilot-scale (Meullemiestre et al., 2015). A lower recovery of capsaicinoids from chilli peppers on a pilot-scale (20 L tank) was obtained using UAE compared to hot maceration at industrial scale, although reductions in temperature and time were achieved (Boonkird et al., 2008).

Pilot-scale studies of UAE directly from soybeans has not been previously reported in the literature. Here the effects of ultrasound on the protein extraction yield during soybase production (pilot-scale) are shown using lab and pilot-scale probe systems. It was hypothesised that an improvement in extraction yield, as found before at lab-scale (Preece et al., 2017b), will be observed at pilot-scale as well due to the improved availability of of protein in the aqueous phase. A central composite design (CCD) was employed to examine the effects of okara concentration, okara flow rate and temperature of ultrasound treatment on the protein extraction yield at pilot-scale versus a conventional method for extraction. The optimum conditions are identified for the specific conditions tested and analysis of variance (ANOVA) will be employed to determine the significance of factors. Particle size measurements and an examination of the microstructure of the materials are performed to aid in identification of the mechanisms of ultrasound.

2. Materials & methods

2.1. Sample production

Soy slurry and okara were prepared from commercially available soybeans using pilot plant facilities (Unilever Research & Development, Vlaardingen). A process flow diagram and stream information can be seen in Fig. 1 and Table 1. Under these processing conditions, it was possible to prepare soy slurry and okara to test the effects of ultrasound. Soybeans (stream 3, Fig. 1) went through two wet milling stages to produce a soy slurry under alkaline conditions. The processing input consisted of 28 kg h^{-1} of soybeans treated with 175 kg h^{-1} of softened water and 0.2 kg h^{-1} of sodium bicarbonate. To prepare soybase and okara for subsequent treatment, the slurry was fed into a decanter centrifuge operating at a *g*-force-time of 1.5×10^5 *g*-s. Table 2 shows the average compositions of okara (Fig. 1, stream 8) and soy slurry (Fig. 1, stream 4) produced using the pilot plant processing equipment *without ultrasonic treatment*.

Fig. 1 also shows the process flow diagram for:

(i) & (ii) *Lab-scale ultrasonic treatment* of the okara and slurry from pilot-scale production (see section 2.2). Here, materials were transferred from the pilot plant and treated with a bench-scale 400 W probe previously used to study lab-scale extraction (Preece et al., 2017b).

(ii) *Pilot-scale ultrasonic treatment* of okara, where the okara was treated using a pilot-scale 2000 W probe (see section 2.3).

A schematic diagram of both the lab and pilot-scale probe systems can be seen in Fig. 2.

2.2. Laboratory–scale sonication

A bench-scale batch ultrasound probe system (Branson Sonifier 450, Branson Ultrasonics Corporation, Danbury, CT), (20 kHz, 65 W (output according to manual), 13 mm probe tip) was utilised to study the effects of ultrasound on slurry and okara solution samples produced in the pilot plant. The lab-scale probe is described schematically in Fig. 2A. Ultrasound treatment times from 0 min (control) up to 15 min were investigated to vary the energy input to the system. After the sample was treated for the desired time, the sample was immediately centrifuged at 4330 × *g* for 10 min.

The energy input was calculated using the equations reported by Bates and Patist (2010), which gives a unit independent of the scale of treatment for continuous and batch operation:

$$\textit{Energy input},\ W_{\text{input}} = \frac{P\ (\text{kW})}{Q\ (\text{L h}^{-1})} = \frac{P\ (\text{W}) \times t\ (\text{s})}{3.6 \times 10^6 \left(\frac{\text{J}}{\text{kWh}}\right) \times V\ (\text{L})} \tag{1}$$

where power (*P*) was calculated using the details from the supplier, and *Q* is the volumetric flow rate for continuous application. Power (*P*), time (*t*) and volume (*V*) were necessary to calculate energy based on batch operation. The volume for both slurry and okara solution treatments prepared at lab-scale was 100 mL. The calculation of the energy input for the lab-scale system was necessary in order to select the energy input range to be studied at pilot-scale.

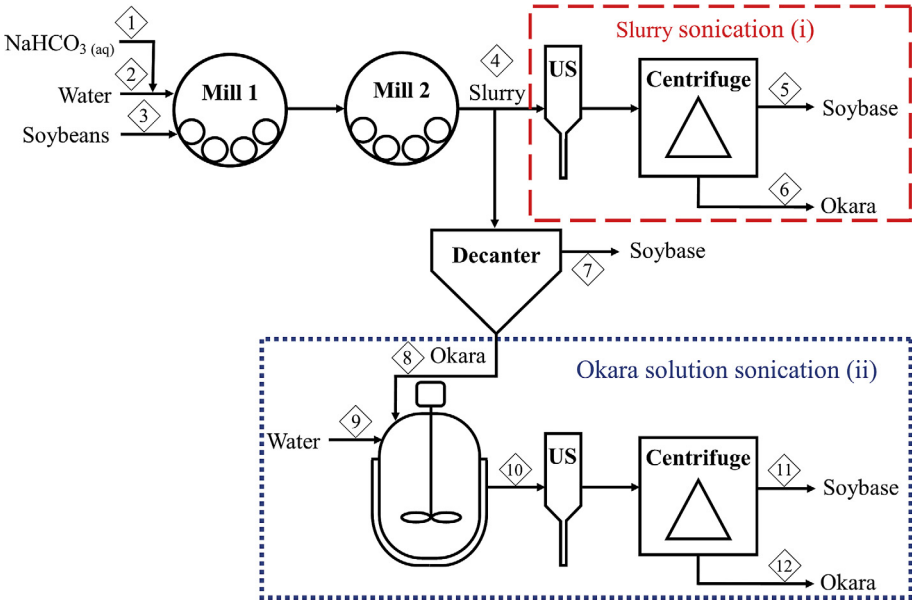

Fig. 1. Process flow diagram of pilot plant slurry preparation and ultrasound treatments of slurry and okara solution samples. The red box (— —) shows the flowsheet for slurry sonication, only investigated at lab-scale. The blue box (···) shows the process for okara solution sonication, carried out at lab & pilot-scale. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Mass flow rates of components and temperatures of streams described in Fig. 1.

Stream	1	2	3	4	7	8
T (°C)	25	85	25	85	–	–
$\dot{m}_{protein}$ (kg h^{-1})	0	0	10.3	10.4	7	3.2
$\dot{m}_{oil}$ (kg h^{-1})	0	0	5.5	5.6	3.3	1.8
$\dot{m}_{moisture}$ (kg h^{-1})	8.9	163.6	2.4	174	126.2	48.2
$\dot{m}_{other}$ (kg h^{-1})	0.2	0	10	10	4.3	6
$\dot{m}_{total}$ (kg h^{-1})	9.1	163.6	28.2	200	140.8	59.2

Table 2
Average okara and slurry composition produced without ultrasonic treatment. The error shown is the deviation in production over 5 different productions. Stream N° corresponds to the stream number labelled in Fig. 1.

	Percentage (%)	
Stream N°	4	8
Component	**Slurry**	**Okara**
Protein	5.2 ± 0.1	5.4 ± 0.2
Oil	2.8 ± 0.1	3.1 ± 0.3
Moisture	87.2 ± 0.2	81.4 ± 0.5
Other	4.8 ± 0.2	10.1 ± 0.6

2.2.1. Soy slurry treatment

Soy slurry (100 g) from pilot-scale production (Fig. 1, stream 4; i.e. prior to separation in the decanter; for composition, see Table 2) was heated to 80 °C using a hot plate and stirred using a magnetic stirrer bar set to a speed of 200 rpm (25 mm in length, 10 mm in diameter cylindrical bar). Once the desired temperature was reached after approximately 15 min, the sample was transferred to a jacketed vessel and the ultrasound treatment was commenced with temperature control. A temperature of 80 °C was chosen for soy slurry treatments as this is the temperature the slurry would be after production in the pilot plant/factory line.

2.2.2. Okara solution treatment

Okara produced in the pilot plant facilities (Fig. 1, stream 8; for composition, see Table 2) was diluted approximately 7 times to an okara concentration of 13.7% (w/w, solid content about 2.5 ± 0.1%) using demineralised water. A sample size of 10 g of okara solution was heated to 50 °C using the same method described in Section 2.2.1. Temperature control was also employed during okara solution sonication by counter-current flow of cool water in a jacketed vessel. For okara solution a temperature of 50 °C was used as this was the resulting temperature after the addition of water to pilot-plant produced okara at 80 °C.

2.3. Pilot-scale sonication of okara solution

To examine the effects of ultrasound on a larger scale, samples were prepared and treated using continuous ultrasonic pilot-scale equipment. When choosing parameters for investigation, the energy input (kWh L^{-1}) was calculated for the lab scale sonication system using Equation (1) (using input information from the supplier) and scaled to a larger scale probe ((UIP2000hd, Hielscher Ultrasonics GmbH, Germany) (20 kHz, 2000 W, 100% amplitude) fitted with booster B2-1.4 and sonotrode CS2d40L3) (Bates and Patist, 2010). This pilot-scale probe is described in Fig. 2B.

Once produced, okara was diluted to the desired concentration using water in a tank stirred at 600 rpm suitable for heating (100 L capacity). Okara solution was pumped using a positive displacement pump through a jacketed ultrasonic flow cell; the jacketed vessel was employed to reduce heating of the okara solution samples during ultrasonic treatment. A counter-current flow of cold tap water was employed; an approximate flow rate of water of 10 kg h^{-1} was recorded. For each trial, a sonicated okara solution sample was collected and also a control sample which passed through the same set-up, with the ultrasound switched off. Samples were then centrifuged in a bench top centrifuge (4330 × *g*, 10 min) and analysed for their compositions in order to calculate extraction yields.

2.4. Oil, protein and solids determination and extraction yield calculations

To examine the effects of ultrasound, extraction yields for oil,

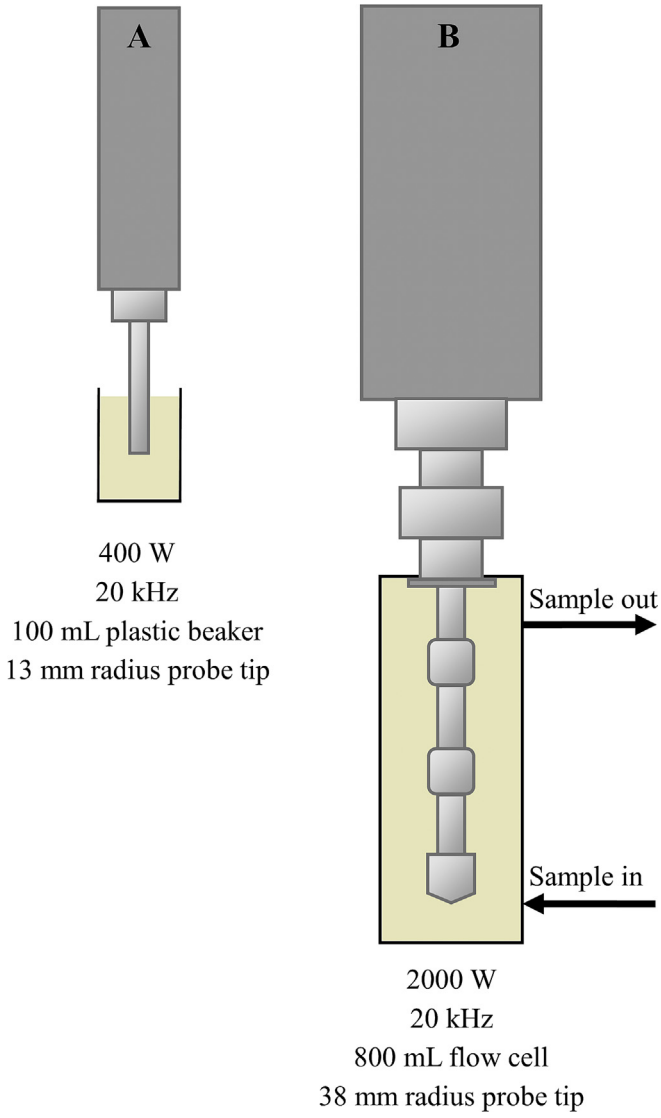

Fig. 2. Schematic diagram of probe sizes for the lab-scale (A) and pilot-scale (B) probe systems.

protein and solids were calculated from the relevant concentrations in the process streams.

To determine protein extraction yields, the protein content on a wet basis (w.b.) was defined in the pellets and supernatants using the Dumas method (Vario MAX CNS, Elementar Analysensysteme GmbH, Germany). L(+)-glutamic acid (VWR International BVBA, Belgium) was used as a standard sample and UHT milk (3.5% fat) (muva kempten, Germany) as a reference material. For soy samples, a protein conversion factor of 6.25 × N was utilised to determine protein content from the measured nitrogen content. From the protein concentrations and masses of streams, the protein extraction yield into the soybase could be calculated using Equation (2):

$$\text{Protein extraction yield} = Y\,(\%) = \left[\frac{S \cdot x_{p,s}}{(S \cdot x_{p,s} + O \cdot x_{p,o})}\right] \times 100 \quad (2)$$

Here S (soybase) and O (okara) represent the total weight of samples and x_p is the mass fraction of protein.

To analyse the effects of ultrasound on okara solution, it was necessary to consider the total protein extraction yield calculated using Equation (3): i.e. the recovery from both slurry and okara solution. During production of soybase including treatment of the okara, two extraction occur in series; the primary extraction from soybeans to soybase (stream 7, Fig. 1) and secondary extraction from okara solution to soybase (stream 11, Fig. 1). Yield Y_I refers to the primary extraction producing soybase and okara (streams 7 & 8, Fig. 1); yield Y_{II} corresponds to the extraction from okara solution (streams 11 & 12, Fig. 1).

$$\text{Total protein extraction yield}\ (\%) = Y_I + (100\% - Y_I) \times Y_{II} \quad (3)$$

Oil and solid contents were measured using a microwave moisture analysis system equipped with NMR for direct detection of fat content (SMART System5, CEM GmbH, Germany). Oil and solid extraction yields were also determined using Equation (2), replacing the masses of protein, with the respective masses. For the analytical measurements of oil, protein and solids, every sample was measured in duplicate and an average was calculated. A pooled standard deviation (SD_{pooled}) was calculated for each method of analysis based on the measurement of 159 samples in duplicate: values of 0.01%, 0.04% & 0.06% were calculated for oil, protein and solids, respectively.

2.5. Experimental design

Experiments for the pilot-scale were defined using response surface methodology (RSM) to test the importance of three input variables: okara concentration (X_1), okara solution flow rate through the ultrasonic flow cell (X_2) and temperature (X_3) on the output response: protein extraction yield. A central composite design was made using the software JMP (v11 from SAS, Cary, NC), including measurement of the centre point twice, totalling 16 trials. The input variables and randomised trial order can be found in Table 3. All input variables were tested on 5 levels ($-\alpha$, -1, 0, $+1$, $+\alpha$) with an α-value of 1.287. The upper and lower levels were set based on limitations of the pump (flow rate and okara concentration) and sensible temperature values. An analysis of variance (ANOVA) was performed once results were gathered to determine the significance of the three parameters tested.

2.6. Particle size measurement

The particle sizes of soy slurries after extraction were determined using laser diffraction (Mastersizer 2000 Hydro S, Malvern Instruments Ltd, UK). To determine particle size distributions (PSDs), refractive indices of 1.33 and 1.45 were used for the water and the particles, respectively (Preece et al., 2017b). Protein, moisture and particle sizes were measured in triplicate for each sample.

2.7. Confocal laser scanning microscopy (CLSM)

A Leica TCS-SP5 microscope in conjunction with a DMI6000 inverted microscope (Leica Microsystems Inc., Germany) was used with the dye acridine orange (Polysciences Inc., Warrington, PA) for visualisation using of the effects of ultrasound treatment. One drop of dye stock solution (1% w/v acridine orange) was added to 1–1.5 mL of sample and mixed well before adding the sample to the glass slide. Table 4 shows the lasers utilised for excitation and the corresponding colours assigned to the emission channels.

2.8. Comparison of lab-scale and pilot-scale probes

When studying the effects of ultrasound, it is important that the power input is calculated, rather than quoting values from the

manufacturer. Several factors affect the actual power supplied to the system by ultrasound, such as the age of the probe tip (Vinatoru, 2015). For both probe systems, a calorimetry study was carried out in which the temperature increase was measured during ultrasound treatment of demineralised water. For the lab-scale probe, a sample of 0.1 kg was studied, compared to the pilot-scale probe used to treat a 0.8 kg water sample. The mass of water studied at pilot-scale was chosen due to the volume of the pilot-scale flow cell. Assuming all of the energy supplied to the system by ultrasound (Q) was converted to heat, it can be calculated as:

$$Q = mC_P\Delta T \tag{4}$$

where (m, kg) is the mass of the sample, C_P is the specific heat capacity (4181 J kg^{-1} K^{-1}) (Sicaire et al., 2016) and ΔT is the temperature increase.

To assess the scalability of ultrasound, it was possible to calculate parameters independent of scale. Energy intensity (EI) and acoustic energy density (AED) were calculated using equations from Tiwari and Mason (2012), shown in Equations (5) and (6).

$$\text{Energy intensity, } EI = \frac{4P}{\pi D^2} \tag{5}$$

where P is the power and D is the diameter of the probe tip.

$$\text{Acoustic energy density, } AED = \frac{P}{V} \tag{6}$$

where P is the power and V is the volume of sample treated.

3. Results & discussion

3.1. Laboratory-scale sonication

Fig. 1 shows the process flow diagram for the production of soy samples. To determine the potential for ultrasound-assisted extraction (UAE), it was performed (i) on the slurry from the primary production (stream 4, Fig. 1) and (ii) diluted okara solution (with a solid content of 2.5 ± 0.1% w/w) prepared on pilot plant scale (stream 10, Fig. 1) using a lab-scale probe system. The effects of ultrasound on the oil, protein and solid extraction yields from soy slurry can be seen in Fig. 3. An improvement was observed for each component upon ultrasound treatment, and after 15 min of ultrasound treatment (relative values compared to the 0 min control sample) was:

- Protein extraction increase of 7% (from 66 to 70%)
- Oil extraction enhancement of 19% (from 62 to 74%)
- The yield of solids increased by 10% (from 55 to 60%)

The temperature was held at 80 °C throughout the experiment for slurry treatment. As a control a slurry sample was heated to 80 °C and held at this temperature for 15 min whilst stirring (no ultrasound treatment). No difference in extraction yield of protein, oil or solids was observed, as apparently equilibrium had been reached at the zero time point (data not shown).

Ultrasound was also used to treat a solution containing the okara waste stream from soymilk production (stream 8, Fig. 1). An improvement in extraction yields was observed for all of the components examined during ultrasonic treatment to the okara solution, seen in Fig. 4. A relative improvement of 40% in extraction of the protein available in the okara using ultrasound after 15 min treatment was observed, compared to the 0 min sample (Fig. 4). Oil extraction was increased by 43% after 15 min US treatment relative to the control sample. Extraction of solids found within okara was enhanced by 28% with 15 min ultrasonic treatment relative to the 0 min control. When considering the total protein extraction yield, Y_{II} calculated using Equation (3), a relative increase of up to 6% was observed during okara solution US treatment (84% versus 89%: control (no US) and 15 min treatment, respectively). To understand the mechanism behind this improvement, the separation efficiencies and availability of protein were calculated using equations presented by Preece et al. (2017b). It was found that during okara solution sonication, blue box ii, Fig. 1, the separation efficiency remained constant at a value of 80% throughout the control and all treated samples. However, when studying the availability of protein, a stepwise improvement was seen: an increase of up to 41% was observed during okara solution US treatment (58% versus 82%: control (no US) and 15 min treatment, respectively). Thus, ultrasound caused an improvement in protein extraction yield by solely improving the protein availability, not accompanied by the deliquoring of okara, as has been previously stated for ultrasound treatment of another soybean extract system (Preece et al., 2017b).

Table 3
Experimental domain of central composite design for pilot plant study.

X_j	Uncoded	Lower level	Upper level
Okara concentration (%)	X_1	14	50
Flow rate (kg h^{-1})	X_2	20	100
Temperature (°C)	X_3	50	85

Trial	Input variables			Experimental protein extraction yield (%)	
	X_1	X_2	X_3	Control	Ultrasound
1	32	60	68	42	51
2	46	91	81	40	42
3	46	29	81	40	46
4	18	29	81	58	64
5	32	20	68	40	44
6	14	60	68	59	56
7	50	60	68	42	44
8	32	60	50	43	45
9	32	100	68	38	51
10	18	91	54	51	54
11	18	29	54	51	58
12	32	60	85	42	46
13	18	91	81	41	47
14	46	29	54	41	45
15	32	60	68	41	43
16	46	91	54	39	38

3.2. Pilot-scale sonication

Positive effects of ultrasound had been observed at bench scale, it was important to explore the scale-up opportunity of the technology at pilot-scale.

An overview of the energy inputs of the lab-scale and pilot-scale probe systems for slurry and okara solution treatment are presented in Table 5. Energy inputs were calculated using Equation (1) that allows a comparison between the systems energy inputs, independent of scale. To achieve a similar energy input for slurry treatments found at lab-scale, up to 19 passes of slurry were required through the pilot-scale flow cell (0.8 L) at the operating flow rate of 200 kg h^{-1} found during its pilot-scale production (Table 5). A single pass through the flow cell could be achieved using the equipment available; however, the energy input would have been quite limited and recirculation of the material through the ultrasonic field was not experimentally feasible. Soy slurry sonication (slurry sonication (i), Fig. 1) with multiple probes in series was not considered as there was access to only one large scale probe system. Large changes in the soy slurry flow rate were not possible due to the throughput of the mills in the pilot plant.

Table 4
CLSM excitation and emission settings specified for acridine orange.

Fluorochrome	Excitation wavelength (nm)	Emission wavelengths (nm)	Representing colour in micrographs
1% w/v Acridine orange	488	497–556	Green
	561	569–646	Red
		655–724	Blue

Therefore, only the okara solution was investigated at pilot-scale (okara solution sonication (ii), Fig. 1) as its flow rate could be adjusted independently of the slurry flow rate for suitable energy inputs similar to those obtained at lab-scale (see Table 5). The flow rate of the okara solution through the ultrasonic cell was varied using a pump operating within the range 20–300 kg h^{-1}.

An alternative technique for slurry treatment based on the phenomena of cavitation is high pressure homogenisation (HPH). HPH improved the protein extraction yield from soy slurry by 25% by a single pass at 100 MPa (Preece et al., 2017a). Industrial scale homogenisers are commercially available that are capable of processing at high pressures for the desired flow rates of soy slurry, offering a suitable alternative to ultrasound (www.gea.com/en/products/homogenizers-one-series.jsp).

3.2.1. Experimental design studies

There are a large number of factors that affect the outcome of ultrasonic treatment (Soria and Villamiel, 2010), therefore design of experiments was employed to test a large investigational area. Three important variables were examined using a central composite design (CCD) in this instance: okara concentration (X_1), okara flow rate (ultrasonic duration, X_2) and temperature of treatment (X_3). Table 3 shows the response variable (experimental protein extraction yield) and how this responds to different processing conditions related to ultrasonic treatment. The upper and lower levels of the input variables okara concentration and okara solution flow rate were pre-selected based on the limitations of the available pump system and the solution viscosity. Okara solution flow rate levels were selected based on a comparison between the bench-scale and pilot-scale probe systems. An analysis of variance (ANOVA) was carried out to determine the significance of the studied variables as well as the ultrasonic treatment itself. All terms were examined for linear, quadratic and interaction effects. Results of the ANOVA from the model can be seen in Table 6. Factors were considered to be significant at a confidence level of 95% (p-value < 0.05). Temperature was excluded from the model, as its effects were not found to be significant (p-value = 0.8858) in the range investigated (50–80 °C). The insignificance of this factor is due to the previous heat treatment during okara production (85 °C milling of soybeans). Even though the solubilisation time is low during the milling stages and prior to separation, the residual heat after production is high, therefore denaturation of protein is possible. There are no further effects of heating the okara solution to 85 °C compared to 50 °C. It was found that the linear effects of input variables examined were significant, as well as the effects of ultrasound treatment and the quadratic effect of okara concentration (X_1^2) (Table 6). Interactions between input variables were not found to be significant, therefore no synergistic effects were observed (p-value > 0.05).

With the data obtained from the CCD, it was possible to deduce a relationship between the variables and the protein extraction yield. This relationship can be seen in Equation (7) containing only the significant factors:

$$Y_{II} = 43.7 - 7.3X_1 - 2.56X_2 + 2.1(US) + 5.9X_1^2 \quad (7)$$

where Y_{II} represents protein extraction yield, X_1 is the okara concentration, X_2 is the okara solution flow rate through the ultrasonic flow cell and US corresponds to ultrasonic treatment ($US = +1$ for ultrasound, $US = -1$ for control experiment). The model has a reasonable fit to the variability of the data ($R^2 = 0.791$) obtained from these pilot plant trials. The adjusted R^2 value is corrected for the number of input parameters investigated in the model; the value presented ($R^2_{adj} = 0.741$) shows a good correlation between observed and predicted data. Equation (7) shows that ultrasonic treatment improves the protein yield (Y_{II}) by 4.2% (difference between 2.1 × +1 for ultrasound and 2.1 × −1 for control), independent of okara solution flow rate and okara concentration.

Previous pilot-scale studies (Meullemiestre et al., 2015; Moulton and Wang, 1982; Pingret et al., 2012) achieved a greater extraction yield of the desired component, except for Boonkird et al. (2008), where reductions in time and temperature compared to conventional extraction methods were observed. In the present study, a significant improvement in yield is observed, yet not as great as those found previously from ultrasonic treatment of slurry and okara solution prepared at lab-scale (see Section 3.1 and Preece et al., 2017b).

3.2.2. Response surface plots of ultrasound-assisted extraction (UAE) at pilot-scale

According to the model, the greatest effects on the protein

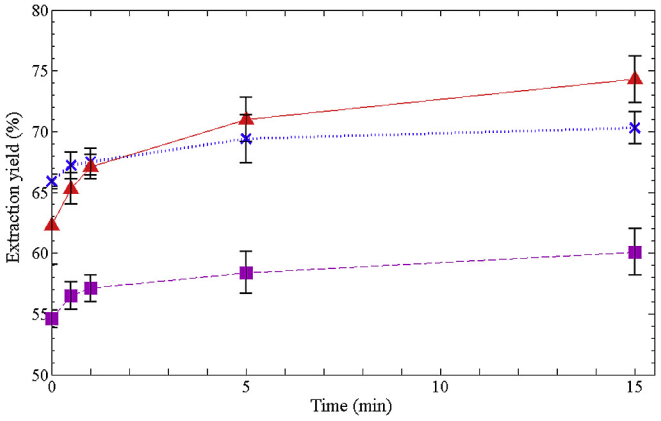

Fig. 3. The effect of lab-scale batch ultrasonic treatment time on the extraction yields of oil (▲), protein (X) and solids (■) from soy slurry (streams 5 & 6, Fig. 1). Error bars show standard error of 3 mean values.

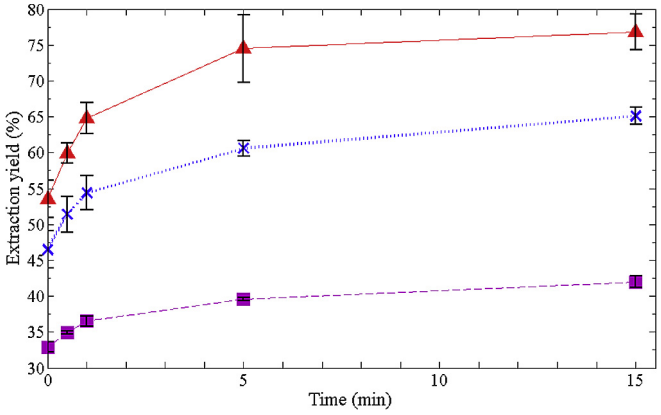

Fig. 4. Extraction yields of oil (▲), protein (X) and solids (■) from okara solution versus ultrasonic treatment times using lab-scale probe system (streams 11 & 12, Fig. 1). Error bars represent standard error of 3 mean values.

Table 5
Energy inputs of the batch lab-scale probe system and the pilot-scale ultrasonic flow.

Lab-scale ultrasonic treatment					
Residence time (s)	0	30	60	300	900
Lab-scale energy input (kWh L^{-1})	0	0.005	0.011	0.054	0.163
Pilot-scale ultrasonic treatment					
Residence time needed for same energy input as at lab-scale (s)	–	9	18	92	275
Pilot-scale ultrasonic treatment of slurry					
Actual residence time (at slurry flow rate 200 kg h^{-1}) (s)	14	14	14	14	14
Number of passes through flow cell	–	0.6	1.3	6.4	19.1
Pilot-scale ultrasonic treatment of okara solution					
Number of passes through flow cell	1		1		1
Flow rate (kg h^{-1})	100		60		20
Actual residence time (s)	29		48		144
Actual energy input (kWh L^{-1})	0.017		0.028		0.085

Please see Fig. 2 for a schematic overview of the probes used in this study. The lab-scale power of probe was quoted as 65 W on the selected setting (according to manufacturer, Branson Sonifier 450). Power of pilot-plant probe system assumed to be 1700 W, based on 85% efficiency (Hielscher UIP2000hd).

extraction yield at pilot scale came from the okara concentration and the okara flow rate, and not from the ultrasound treatment. The effects of the variables on control samples without ultrasound are visualised in Fig. 5. From this plot, it is possible to examine the effects of okara concentration and flow rate on the output variable of interest for the control sample without ultrasonic treatment. The okara concentration is decided by the solid-liquid ratio which is an important parameter when studying solid-liquid extraction. At the lowest okara concentration, there was more solvent available per unit of protein to be extracted. At the highest okara concentration of 50%, there was less solvent available per unit of protein to be extracted and the viscosity increased due to the increased solids, therefore a lower protein extraction yield was observed.

When considering the okara flow rate, it is vital to consider the residence time within the system as well as fluid mechanics, even for the control sample which travels the same path as the treated sample, but without the ultrasonic field. At the lowest tested okara solution flow rate, the protein extraction yield was not optimal, although the residence time was 6 min (Table 7). Another important factor to consider is the flow pattern of the sample and to do this, the Reynolds number (*Re*) was calculated for each flow rate studied using dynamic viscosity data from the literature (Table 7) (Poling et al., 2008). As the flow rate increases, the residence time and the contact time between the extraction medium (alkali water) and components, such as protein, will decrease. When considering the flow regime of the solution through the piping, it has been calculated that the flow was laminar at 60 kg h^{-1} and below, with the motion of fluid always following fluid streamlines (Table 7). For okara solution flow rates of 91 and 100 kg h^{-1}, the flow is in a transition region and this corresponds to less regular flow caused by irregular transverse eddies. From the studied conditions, a maximal extraction yield was observed at a mid-range of okara solution flow rate; this was probably caused by a balance between fluid dynamics and solubilisation times. The highest yield was 59% without ultrasound (at 14% okara concentration, 60 kg h^{-1} okara solution flow rate, temperature of 68 °C) and 64% when ultrasound was added (at 18% okara concentration, okara solution flow rate of 29 kg h^{-1}, and temperature of 81 °C, Fig. 6). The maximum effects observed experimentally are different for okara solution washing versus okara solution sonication.

Fig. 6 can also be used to confirm the infeasibility of ultrasonic treatment of slurry at the fixed flow rate of 200 kg h^{-1}. At an okara solution flow rate of 100 kg h^{-1}, it can be seen that a plateau has already been reached and no effects of ultrasound can be observed on the protein extraction yield (Fig. 6). This verifies the decision not to explore slurry treatment at pilot scale due to the limited energy input by the pilot-scale probe system compared to the lab-scale probe.

3.2.3. PSD

One important characteristic of the sample which changes as a result of ultrasonic treatment is the PSD. It is well documented that ultrasonic processing results in a reduction in particle size. These effects on resulting supernatant (stream 11, Fig. 1) can be seen in Fig. 7. In the control sample, the soybase obtained after centrifugation of okara solution without ultrasound treatment had a broad particle size distribution with particles up to 10 μm present. Ultrasonic treatment at all okara solution flow rates investigated resulted in a reduction of the particle size of the corresponding soybases. A stepwise reduction in the particle size can be observed. The lowest flow rate of 20 kg h^{-1}, corresponding to the longest residence time in the ultrasonic field, yielded particles up to 1 μm in size. Reducing the particle size of the resulting soybase may improve the storage stability of the final soy beverage products.

3.2.4. CLSM

To understand the effects of ultrasound, the microstructure of processing materials were investigated using CLSM. Fig. 8 shows

Table 6
ANOVA for quadratic model of relationship between independent input variables (X_1, X_2, X_3 and ultrasonic treatment) and output (Protein extraction yield (%). Significant sources (p-value <0.05) are highlighted as bold.

Source	Sum of Squares	DF	Mean square	F Value	Prob > F
Model	1187.15	6	197.86	15.76	**<0.0001***
X_1	734.66	1	734.66	58.52	**<0.0001***
X_2	89.65	1	89.65	7.14	**0.0131***
Ultrasonic treatment	142.23	1	142.23	11.33	**0.0025***
X_1^2	140.35	1	140.35	11.18	**0.0026***
X_1*X_2	41.02	1	41.02	3.27	0.0827
X_2*X_3	39.24	1	39.24	3.13	0.0893
Lack of fit	278.34	23	12.10	0.68	0.7488
Pure error	35.53	2	17.76		
Total error	313.86	25			
C.Total	1501.02	31			
R^2	0.791				
R^2_{adj}	0.741				

representative micrographs of okara solution prior to ultrasonic treatment at various magnifications visualised using acridine orange. It is possible to visualise fibrous materials, protein and other biopolymers within these samples (Preece et al., 2015). In Fig. 8A, many features are highlighted in purple (a combination of blue and red emission); these correspond to empty cell wall structures or fibrous material. Green features under these visualisation conditions highlight the more hydrophobic regions and here represent intact soybean cell walls (Fig. 8B and C). Within these cells it is possible to visualise intact protein bodies (appearing green, Fig. 8C) and the cytoplasmic network containing oil bodies stabilised by oleosins are highlighted in purple. These findings are similar to those found by Preece et al. (2015) when visualising soy slurry and okara. It was not possible to locate insoluble protein bodies in the size range 5–20 μm in okara solution samples prior to treatment with ultrasound. These insoluble protein bodies found outside of the cellular matrix were observed in soy slurry and okara that were prepared in the lab (Preece et al., 2015). Apparently, aggregation of protein bodies during pilot plant production does not take place or they reduce in size immediately, most likely due to shorter residence times at high temperature and more efficient milling.

The okara solution processing materials were also investigated with CLSM following ultrasonic treatment (Fig. 9). After treatment of 13.7% okara solution with a flow rate of 20 kg h^{-1} at 50 °C, the microstructure of the ultrasound treated samples were unchanged. It was possible to see that under the tested conditions, ultrasound did not cause intact cells to disrupt, as was also found previously at lab-scale (Preece et al., 2017b).

There are several factors that could be responsible for the presence of intact cells after ultrasonic treatment. The concentration of intact cells within the okara solution is low and therefore the spatial mismatch in liquid jets, a result of cavitation, and intact cells makes disruption less inclined. The action of cavitation will be relatively localised close to the tip of the ultrasonic probe. The force required to disrupt the cell wall of a soybean cell can also be greater than that supplied by liquid jet impingements. In contrast to lab-scale extraction (Preece et al., 2017b), there was also no visible sign of aggregated protein outside intact cells during pilot-scale extraction before UAE. The microstructural analysis undertaken in a previous lab-scale study indicates a reduction in the concentration of aggregated protein as the main cause of the improved yields upon ultrasound treatment (from 47 to about 72% after 15 min), and not cell disruption as is frequently stated in the literature (Preece et al., 2017b). As aggregated protein bodies were not found in the

Table 7
Okara solution mass flow rates and corresponding flow parameters through the piping and flow cell, without ultrasonic treatment. Reynolds number was calculated for okara solution, which behaves as a Newtonian fluid (data not shown).

Mass flow rate (kg h^{-1})	20	29	60	91	100
Residence time (min)	6	4	2	1	1
Reynolds number (*Re*)	732	1062	2197	3332	3662
	Laminar	Laminar	Laminar	Transition region	

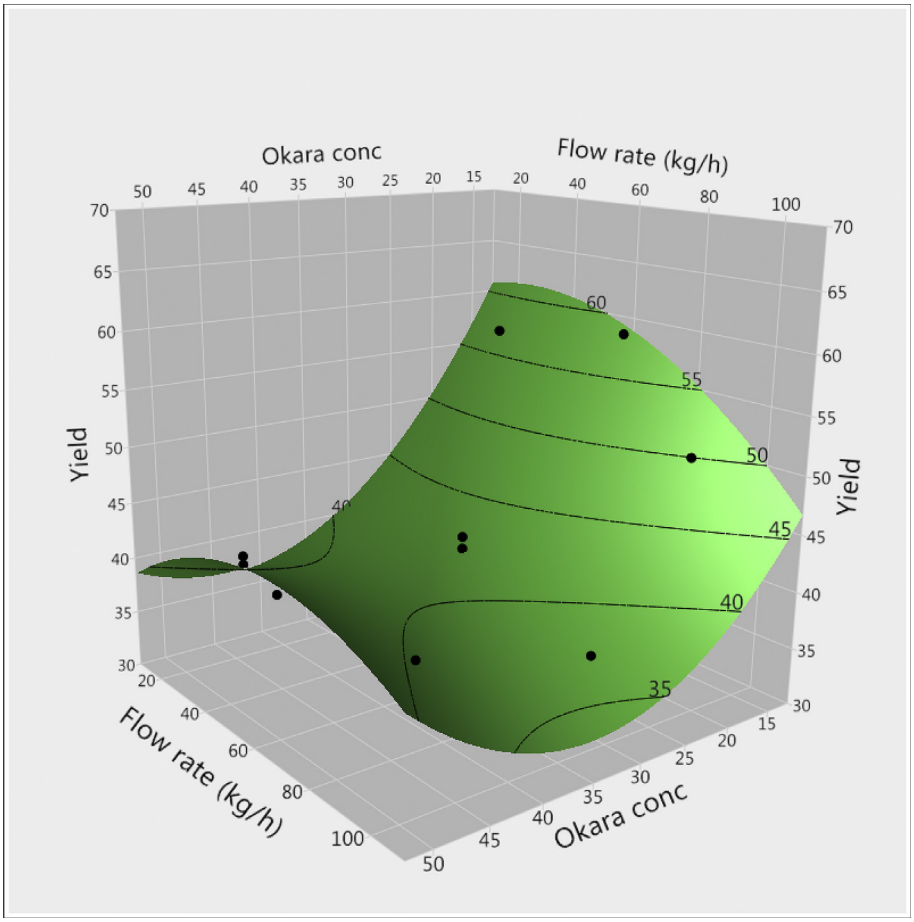

Fig. 5. Response surface plot for protein extraction yield (Equation (2)) as a function of okara concentration and okara flow rate through the ultrasonic flow cell at 68 °C for control sample at pilot-scale (without ultrasound). Black dots are experimental points.

current pilot-scale study, it is proposed that the mechanism for the reduced increase in protein extraction yield of the current study (4.2%) can be attributed to the effects of cavitation on the fibres. Some proteins are available for extraction located in the extracellular material, but are bound to fibres of the cell walls of disrupted cells and subsequently enter the okara waste stream. The energy input from ultrasound is sufficient to overcome the forces binding the protein to the fibrous cell wall materials thus improving the availability of protein.

3.3. Lab-scale and pilot-scale probe comparison

To investigate the differences between lab-scale and pilot-scale sonication, a calorimetry study was undertaken. Details of the experimental method and equations can be found within Section 2.8. To compare the two systems utilised in this study energy intensity (*EI*), acoustic energy density (*AED*) and the measured energy input (W_{input}) are presented (Table 8). When comparing the acoustic energy densities, the values for both probe systems investigated were comparable. These values give an indication of the power experienced per volume of sample. The energy intensities were vastly different between the systems studied. The intensity of the lab-scale probe was approximately 300 times that of the pilot-scale probe. Energy intensity is the power supplied per surface area of the probe tip. The energy input (W_{input}) ranges studied also had significant differences. Table 8 shows that the pilot-scale probe was unable to offer an energy input of more than 0.03 kWh L^{-1}, which was limited by the lower processing flow rate of the pump for okara solution.

3.4. Viability of ultrasound treatment of okara solution during soymilk production

3.4.1. Oxidation of oil during ultrasonic treatment

It has been previously reported that excessive ultrasound

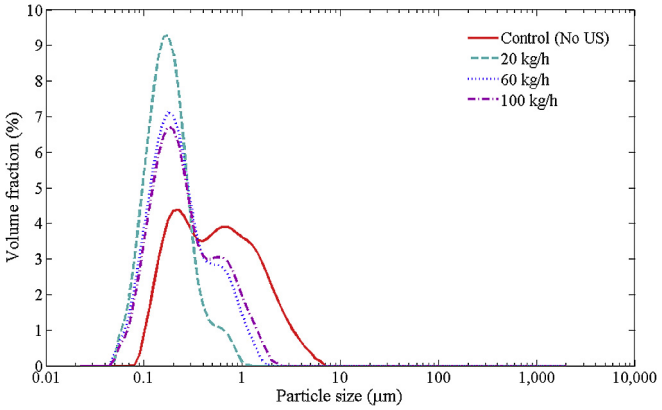

Fig. 7. Effects of okara solution flow rate (longer residence time in ultrasonic field) on the particle size distribution of resulting soybase after centrifugation (stream 11, Fig. 1). Okara solution – no US shows the PSD of the supernatant after separation without ultrasound.

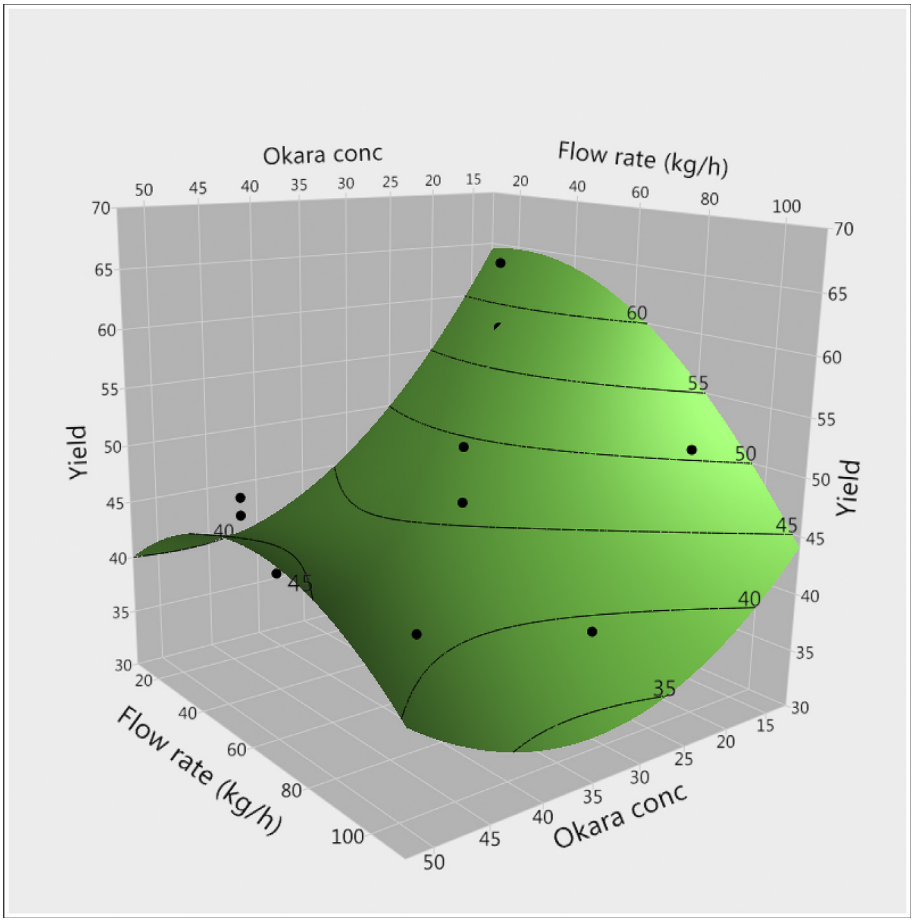

Fig. 6. Response surface plot for protein extraction yield (Equation (2)) as a function of okara concentration and okara flow rate through the flow cell at 68 °C for ultrasonically-treated okara at pilot-scale. Black dots are experimental points.

treatment can cause oxidation of extracted oil in the soybase sample, leading to the generation of a rancid flavour of the final product (Pingret et al., 2013). When considering the implementation of ultrasound at industrial scale, it is important to study the production of degradation products and to compare to the conventional production process. Here, headspace analysis was performed using gas chromatography-mass spectrometry (GC-MS), to detect the evolution of hexanal, one of the most abundant degradation products of oil found within the soybean. There was no difference noted between the hexanal detected from control samples (no US) and those treated with ultrasound at all treatment times for samples treated at pilot-scale (data not shown). Thus, UAE of these samples did not result in oil oxidation.

3.4.2. Productivity of ultrasonic treatment

When considering okara solution UAE within industry, there are two viable options for utilisation of the newly created product stream. The side stream can be utilised for a low-protein product, or it can be recirculated back to the main slurry line, if the final protein concentration is not below the threshold value required for soymilk production. If the okara solution treated with the optimum extraction conditions (14% okara, 20 kg h^{-1} okara solution flow rate, 50 °C) is fed into the slurry line (added to stream 4, Fig. 1), then a final soybase protein concentration of 4.5% can be achieved. This is still considered an acceptable concentration of protein; however, over one hour more than 400 kg of okara solution will be produced, which requires 20 h of side stream ultrasonic treatment. Recirculation of the okara is not considered feasible for this reason, as well as issues associated with recirculation and microbial spoilage (Vong and Liu, 2016).

It was not only important to consider the optimum extraction yield achievable, but also the optimal production process. Fig. 10 shows the effects of okara solution flow rate on the protein extraction yield (PEY, %) and productivity. Productivity is a function of the protein concentration (wt. %) of the resultant soybase (stream 11, Fig. 1) and the okara solution flow rate (kg h^{-1}). Varying the okara solution flow rate with fixed temperature and okara concentration (68 °C, 32%) yielded little difference in resulting protein extraction yield compared to the productivity. However, during the optimisation of UAE at pilot-scale (Section 3.2.2) the okara solution flow rate was shown to be a significant factor by ANOVA, with the lowest flow rate resulting in the highest protein extraction yield. The productivity was at its greatest when the okara solution flow rate was at its highest throughput through the ultrasonic flow cell. Economically, it would be more beneficial to choose a faster flow rate and to sacrifice part of the protein extraction yield with a higher throughput of protein. To achieve the highest improvement in protein extraction yield from soybean processing materials, a cell disruption technique would be better suited, for example, high pressure homogenisation (HPH), which is based on hydrodynamic cavitation (Preece et al., 2017a). Treatments of slurry were also possible with HPH; a single pass through the homogeniser (100 MPa) led to a relative improvement of 24% in the protein extraction yield (Preece et al., 2017a). Cost analysis is also necessary to determine the payback time of such equipment and energy requirements to determine the economic feasibility of scale-up.

When considering soybase production, it was appropriate to calculate the total protein extraction yield (Equation (3)). Until now, only the protein extraction yield has been calculated for the single extraction step studied (Equation (2), slurry or okara treatment). The largest improvement in total protein extraction yield compared to a control sample can be observed in trial 9 (Table 3), where an improvement of 6% was measured (80% versus 85% for control sample and ultrasound treatment of okara solution at pilot-scale, respectively). However, the majority of UAE samples had an additional benefit of 1–2% on the total protein extraction yield compared to the control (washing of okara).

At the highest energy input for pilot-scale sonication, a value of 0.03 kWh L^{-1} (30 kWh m^{-3}) was introduced into the okara solution system. When this value is compared to that of a stirred tank of 0.2–1.5 kWh m^{-3} it is possible to see the energy input is up to 150

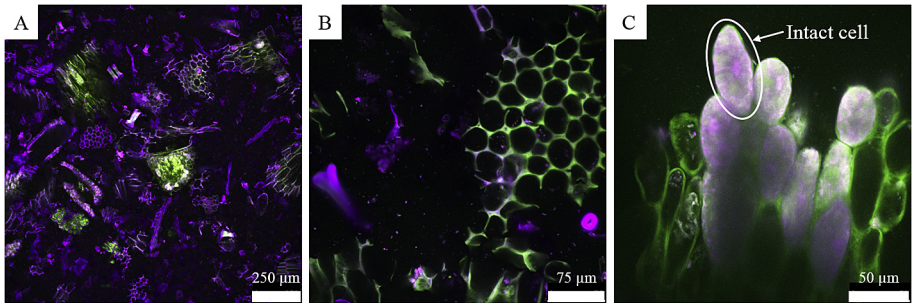

Fig. 8. Representative CLSM micrograph of okara solution prior to ultrasonic treatment. Features are visualised using acridine orange at various magnification levels.

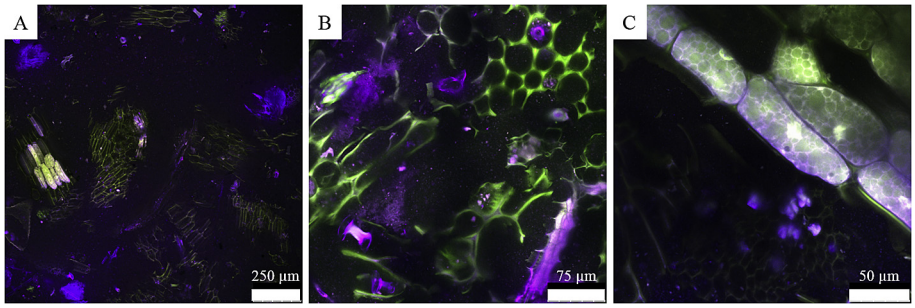

Fig. 9. Representative CLSM micrograph of okara solution after ultrasonic treatment (20 kg h^{-1}okara solution (13.7%) flow rate at 50 °C) observed using acridine orange.

Table 8
Energy intensity (EI), acoustic energy density (AED) and measured energy input range (W_{input}) for both lab (Branson Sonifier 450) and pilot-scale (Hielscher UIP2000hd) probe systems.

	AED (W cm^{-3})	*EI* (W cm^{-2})	W_{input} range (kWh L^{-1})
Branson Sonifier 450 (20 kHz, 400 W, 13 mm probe tip)	0.5	3822	0.004–0.12
Hielscher UIP2000hd (20 kHz, 2000 W, 1.5 inch probe tip)	0.8	13	0.007–0.03

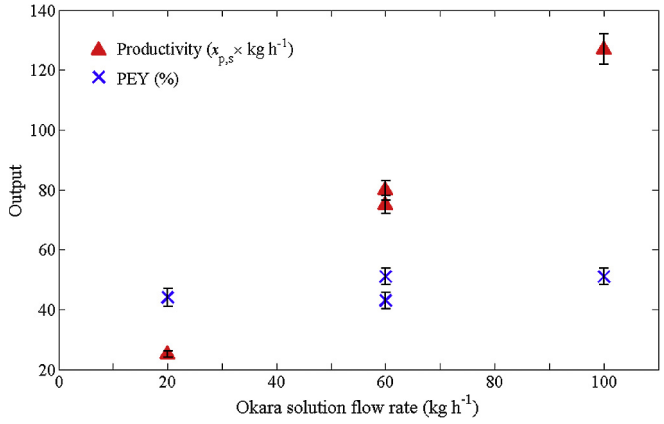

Fig. 10. Productivity and protein extraction yield (PEY) versus okara solution flow rate. Okara concentration and temperature were fixed during ultrasonic treatment (32%, 68 °C). Error bars represent the standard deviation calculated from the SD_{pooled} value. Two points are shown for the flow rate of 60 kg h^{-1} as this was the central point from the experimental design, carried out in duplicate.

times higher for the ultrasonic pilot-scale probe system (Ditl et al., 1992). This should be considered when assessing the viability of the industrial application of ultrasound in any industry.

4. Conclusions

Ultrasound treatment was shown to significantly improve the protein extraction yield by 4.2% during the okara solution treatment on pilot plant scale. Okara solution flow rate and okara concentration also had significant effects on the protein extraction yield. However, considering the whole soybase production process, from soybeans to final processing materials studied, UAE was found to have comparable results to the washing of okara at pilot-scale, contrary to lab-scale sonication. During the lab-scale sonication treatment, a greater energy intensity was experienced by the samples compared to the pilot-scale system, resulting in a greater impact of ultrasound treatment. Okara solution visualised after pilot-scale sonication was found to still contain intact cells, complete with protein bodies inside. No aggregated protein bodies outside the cells were visualised within the starting materials for UAE, therefore a reduced effect was observed in contrast to previous results found for okara prepared at lab-scale. For the extraction of soy protein, one of the world's cheapest and most readily available protein sources, ultrasound is not considered to be the most beneficial unit operation for enhancing the extraction yield, for reasons including the life of the probe and the high energy input.

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References

Aiking, H., 2011. Future protein supply. Trends Food Sci. Tech. 22, 112–120.
Bates, D., Patist, A., 2010. Industrial applications of high power ultrasonics in the food, beverage and wine industry. In: Doona, C.J., Kustin, K., Feeherry, F.E. (Eds.), Case Studies in Novel Food Processing Technologies. Woodhead Publishing Series in Food Science, Technology and Nutrition, Cambridge, pp. 119–138.
Boonkird, S., Phisalaphong, Phisalaphong, C.M., 2008. Ultrasound-assisted extraction of capsaicinoids from Capsicum frutescens on a lab- and pilot-plant scale. Ultrason. Sonochem. 15, 1075–1079.
Chandrapala, J., Oliver, C.M., Kentish, S., Ashokkumar, M., 2013. Use of power ultrasound to improve extraction and modify phase transitions in food processing. Food Rev. Int. 29, 67–91.
Chemat, F., Zill-e-Huma, Khan, M.K., 2011. Applications of ultrasound in food technology: processing, preservation and extraction. Ultrason. Sonochem. 18, 813–835.
Chemat, F., Rombaut, N., Sicaire, A.G., Meullemiestre, A., Fabiano-Tixier, A.S., Abert-Vian, M., 2017. Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. Ultrason. Sonochem. 34, 540–560.
Ditl, P., Rieger, F., Roušar, I., 1992. The design of agitated dissolution tanks. In: Fluid Mechanics of Mixing. Springer, Netherlands, pp. 131–138.
Esclapez, M.D., García-Pérez, J.V., Mulet, A., Cárcel, J.A., 2011. Ultrasound-assisted extraction of natural products. Food Eng. Rev. 3, 108–120.
Jacotet-Navarro, M., Rombaut, N., Deslis, S., Fabiano-Tixier, A.S., Pierre, F.X., Bily, A., Chemat, F., 2016. Towards a "dry" bio-refinery without solvents or added water using microwaves and ultrasound for total valorization of fruit and vegetable by-products. Green Chem. 18 (10), 3106–3115.
Karki, B., Lamsal, B.P., Jung, S., van Leeuwen, J., Pometto, A.L., Grewell, D., Khanal, S.K., 2010. Enhancing protein and sugar release from defatted soy flakes using ultrasound technology. J. Food Eng. 96, 270–278.
Lee, H., Yildiz, G., dos Santos, L.C., Jiang, S., Andrade, J.E., Engeseth, N.J., Feng, H., 2016. Soy protein nano-aggregates with improved functional properties prepared by sequential pH treatment and ultrasonication. Food Hydrocoll. 55, 200–209.
Li, Y., Fabiano-Tixier, A.S., Tomao, V., Cravotto, G., Chemat, F., 2013. Green ultrasound-assisted extraction of carotenoids based on the bio-refinery concept using sunflower oil as an alternative solvent. Ultrason. Sonochem. 20 (1), 12–18.
Li, H., Pordesimo, L., Weiss, J., 2004. High intensity ultrasound-assisted extraction of oil from soybeans. Food Res. Int. 37, 731–738.
Meullemiestre, A., Petitcolas, E., Maache-Rezzoug, Z., Chemat, F., Rezzoug, S.A., 2015. Impact of ultrasound on solid-liquid extraction of phenolic compounds from maritime pine sawdust waste. Kinetics, optimization and large scale experiments. Ultrason. Sonochem. 28, 230–239.
Moulton, K.J., Wang, L.C., 1982. A pilot-plant study of continuous ultrasonic extraction of soybean protein. J. Food Sci. 47, 1127–1129.
O'Toole, D.K., 1999. Characteristics and use of okara, the soybean residue from soy milk production a review. J. Agric. Food Chem. 47, 363–371.
Patist, A., Bates, D., 2008. Ultrasonic innovations in the food industry: from the laboratory to commercial production. Innov. Food Sci. Emerg. Technol. 9, 147–154.
Pingret, D., Fabiano-Tixier, A.S., Bourvellec, C.L., Renard, C.M.G.C., Chemat, F., 2012. Lab and pilot-scale ultrasound-assisted water extraction of polyphenols from apple pomace. J. Food Eng. 111, 73–81.
Pingret, D., Fabiano-Tixier, A.S., Chemat, F., 2013. Degradation during application of ultrasound in food processing: a review. Food Control 31, 593–606.
Poling, B.E., Thomson, G.H., Friend, D.G., Rowley, R.L., Wilding, W.V., 2008. Perry's Chemical Engineers' Handbook, eighth ed. The McGraw-Hill Companies, Inc, New York (Chapter 2).
Preece, K.E., Drost, E., Hooshyar, N., Krijgsman, A., Cox, P.W., Zuidam, N.J., 2015. Confocal imaging to reveal the microstructure of soybean processing materials. J. Food Eng. 147, 8–13.
Preece, K.E., Hooshyar, N., Krijgsman, A.J., Fryer, P.J., Zuidam, N.J., 2017a. Intensification of Protein Extraction from Soybean Processing Materials Using Hydrodynamic Cavitation. Innov. Food Sci. Emerg. Technol. 41, 47–55.
Preece, K.E., Hooshyar, N., Krijgsman, A.J., Fryer, P.J., Zuidam, N.J., 2017b. Intensified

soy protein extraction by ultrasound. Chem. Eng. Process 113, 94–101.
Rosenthal, A., Pyle, D.L., Niranjan, K., 1998. Simultaneous aqueous extraction of oil and protein from soybean: mechanisms for process design. Food Bioprod. Process 76, 224–230.
Shirsath, S.R., Sonawane, S.H., Gogate, P.R., 2012. Intensification of extraction of natural products using ultrasonic irradiations: a review of current status. Chem. Eng. Process 53, 10–23.
Sicaire, A.G., Vian, M.A., Fine, F., Carré, P., Tostain, S., Chemat, F., 2016. Ultrasound induced green solvent extraction of oil from oleaginous seeds. Ultrason. Sonochem. 31, 319–329.
Soria, A.C., Villamiel, M., 2010. Effect of ultrasound on the technological properties and bioactivity of food: a review. Trend Food Sci. Tech. 21, 323–331.
Tiwari, B.K., Mason, T.J., 2012. Ultrasound processing of fluid foods. In: Cullen, P.J., Brijesh, K.T., Vasilis, V., Vasilis, V. (Eds.), Novel Thermal and Non-Thermal Technologies for Fluid Foods. Academic Press, London, pp. 135–165.
Vilkhu, K., Mawson, R., Simons, L., Bates, D., 2008. Applications and opportunities for ultrasound assisted extraction in the food industry - a review. Innov. Food Sci. Emerg. Technol. 9, 161–169.
Vinatoru, M., 2015. Ultrasonically assisted extraction (UAE) of natural products some guidelines for good practice and reporting. Ultrason. Sonochem. 25, 94–95.
Vishwanathan, K.H., Singh, V., Subramanian, R., 2011. Wet grinding characteristics of soybean for soymilk extraction. J. Food Eng. 106, 28–34.
Vong, W.C., Liu, S.Q., 2016. Biovalorisation of okara (soybean residue) for food and nutrition. Trend Food Sci. Tech. 52, 139–147.
Wijngaard, H.H., Zuidam, N.J., 2014. Soybean Extraction Process. International Publication WO14154472.